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# Food Web Assembly at the Landscape Scale: Using Stable Isotopes to Reveal Changes in Trophic Structure During Succession

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## ABSTRACT

Food webs are increasingly evaluated at the landscape scale, accounting for spatial interactions involving different nutrient and energy channels. Also, while long viewed as static, food webs are increasingly seen as dynamic entities that assemble during vegetation succession. The next necessary step is, therefore, to link nutrient flows between ecosystems to local food web assembly processes. In this study, we used a 100-year salt marsh succession in which we investigated the long-term changes in food web organization, especially focusing on the balance between internal versus external nutrient sources. We found that during food web assembly, the importance of internal (terrestrial) nutrient cycling increases at the expense of external (marine) inputs. This change from external to internal

nutrient cycling is associated with strong shifts in the basis of energy channels within the food web. In early succession, detritivores are mostly fuelled by marine inputs whereas in later succession they thrive on locally produced plant litter, with consequences for their carnivores. We conclude that this 100 years of food web assembly proceeds by gradual decoupling of terrestrial nutrient cycling from the marine environment, and by associated rearrangements in the herbivore and detritivore energy channels. Food web assembly thus interacts with nutrient and energy flows across ecosystem boundaries.

**Key words:** stable isotope analysis; succession; ecosystem assembly rules; food web; detritivores; herbivores; salt marsh; chronosequence.

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**Author Contributions:** MS, MPB, and HO developed the ideas for this study. JJ and MS conducted the research and analyzed the data. MS wrote the first draft, which was then edited by MPB and HO.

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## INTRODUCTION

Food web studies have so far revealed many informative and repeatable patterns in trophic structure (for example, Pimm 1982; Cohen and Briand 1984; Cohen and Newman 1985; Dunne and others 2002), body size distribution, and topology (Cohen and others 2003; Woodward and others 2005). In addition, it is increasingly recognized that the understanding of the architecture of many food webs requires the inclusion of above- and belowground

parts of ecosystems, as well as spatial interactions and/or temporal dynamics between food webs (Winemiller and Polis 1996; Moore and others 2004; Berg and Bengtsson 2007; Rooney and others 2008). These authors have shown that food webs are composed of different energy channels based on discrete resources, which are often not homogeneously spread in space and time, thus influencing the different components of the food web in their own way. Moreover, spatial interactions among neighboring ecosystems, such as between ocean and desert, cause some food webs to connect to others on a landscape scale (Winemiller 1990; Polis and Hurd 1995; Rooney and others 2008). Specifically, spatial subsidies into the detrital part of the food web have been shown to have far-reaching consequences for food web structure and ecosystem functioning. They often lead to higher primary production, which in turn may cascade up trophic chains (Polis and Hurd 1995; Polis and Strong 1996).

Although the role of spatial interactions in food web organizations are increasingly studied, good case studies of food web assembly over sufficient time are still poorly available, especially for food webs that are connected to others at the landscape scale. This lack is possibly explained by the large effort required in the quantification of the food web components in the first place, combined with the additional complications involved in following food web structure over sufficient time. One of the most obvious changes in food web structure that explicitly focuses on this temporal component can be found during primary succession. However, succession research has merely focused on vegetation and soil changes (Clements 1916; Miles and Walton 1993). The few notable exceptions (Van de Koppel and others 1996; Kaufmann 2001; Neutel and others 2007) have addressed only parts of food webs, rather than providing an integrated analysis of their structure.

Given the importance of spatial subsidies and temporal changes in food webs for understanding the processes of ecosystem assembly, combining insights from food web ecology on a landscape scale, that is, combining vegetation succession research and food web assembly in single study systems, is now needed. More precisely, we need to find out how different food web components, that is, the green component of food webs, consisting of plants and herbivores and their predators, and the brown component of food webs, consisting of plant litter, detritivores, and their predators interact over a successional sequence by taking the spatial aspects of food webs into account. This can be done by building on recent insights in factors structuring different

energy channels in food webs: specifically the balance between the brown (detritivore-driven) and green (herbivore-driven) parts of food webs (Cebrian and Lartigue 2004; Moore and others 2004; Rooney and others 2006; Shurin and others 2006).

In this study, we used a chronosequence reflecting 100 years of primary succession on a coastal salt marsh to study food web assembly on a landscape scale. Previous work on our study system has quantified in detail the dynamics of soil and vegetation succession (Olff and others 1997) and its interaction with vertebrate herbivores (Van de Koppel and others 1996; Van Wijnen and Bakker 1999; Kuijper and Bakker 2005). This chronosequence has been carefully validated by 35 years of study of permanent plots, which has justified its space-for-time replacement (van Wijnen and others 1997; Schrama and others 2012). This chronosequence approach allowed us to study 100 years of successional dynamics during the same year. We now add analyses of the trophic dynamics, by explicitly focusing on the brown and green part of the food web along the chronosequence to study changes in food web organization over succession. In addition, we use stable isotope analyses to study the landscape origin of the carbon and nitrogen used by different compartments.

Stable isotopes are excellent tools to study temporal changes in food web organization, and nutrient and carbon sources for the different energy channels, especially in coastal environments. Carbon isotopes are useful in discriminating marine from terrestrial sources (Polis and Hurd 1995; Maron and others 2006), especially when the terrestrial plant species are predominantly C3 plants, which is the case in our system. Nitrogen isotopes are useful in studying the trophic position of different groups (Hobson and Welch 1992; Post 2002), and can thus also be used to study changes in trophic position and structure throughout succession.

Specifically, we unravel which nutrient and energy sources fuel the food web in the various stages of vegetation succession. Then we study how the main food web organization in this ecosystem changes over 100 years of primary succession. For this, we combine information on animal diets with measured nitrogen isotope values for each main trophic group in different stages of succession.

## METHODS

### Study Area and Sampling Design

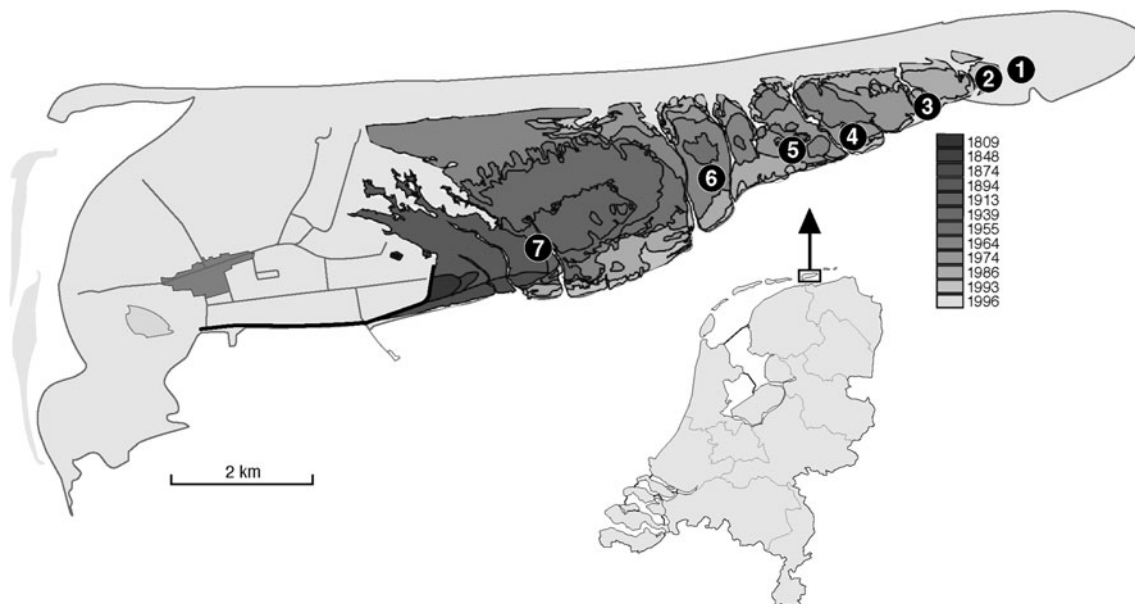
Our study area was located on the island of Schiermonnikoog (53°30'N, 6°10'E), the Netherlands.

A sequence of vegetation succession stages on the salt marsh was present, where the earliest stages are formed on the east side of the island, and later stages of succession were situated 8 km to the west (Olff and others 1997). The sampling locations get inundated regularly at high tides. Marine algae and other organic material wash ashore during these inundations. At high tide, many resting waders are present in the area, especially during spring and autumn migration, but many non-migrating waders are also present during the whole year.

Seven succession stages were identified, estimated as 0, 10, 25, 35, 45, 55, and 100 years of primary succession in 2010 (Figure 1; see Olff and others 1997 for details). Salt marsh age at each successional stage was estimated from topographic maps, aerial photographs, and the thickness of the sediment layer accumulated on top of the underlying sand layer (Olff and others 1997; Van Wijnen and Bakker 1999), and calibrated using long-term observations of permanent plots (van Wijnen and others 1997). The sites were selected to have a similar base elevation (vertical position with respect to mean sea level at the initial elevation gradient on the bare sand flats, before additional sedimentation happened due to vegetation succession). A base elevation of 1.16 m ( $\pm$  SE 2.2 cm) above Dutch Ordnance Level (N.A.P.) was used to

select the sites for this study. Details on every sampling site can be found in the electronic appendix, Table 1. Along our succession gradient, inundation frequency declines due to ongoing sedimentation. This is associated with a gradual decline in the clay and organic matter input along the successional sequence (Olff and others 1997). The earliest stage of succession had an average inundation frequency of 184 times  $y^{-1}$  (26% of all tidal cycles), whereas the last stage of succession has an inundation frequency of 131 times  $y^{-1}$  (18% of all tidal cycles).

At every succession stage (site) five 25-m spaced plots (5 m  $\times$  5 m each) were placed in which the main trophic groups from the green and brown part of the food web were sampled. Between April 23 and May 14, 2010, field collections of all dominant animals, plants, and marine deposits (species that represent >90% of the biomass in their respective trophic group) were done in each of these plots. The changes in abundances of the different species, and their aggregations into trophic groups, are reported in a previous study (Schrama and others 2012). Sampling methods for all trophic groups for the stable isotope analysis are described in the following paragraphs. For all details on the dominant species that were collected and sample size for every species or organic matter source, see electronic appendix, Table 2.



**Figure 1.** Map of the study area on the island of Schiermonnikoog, the Netherlands. Numbers 1–7 refer to the age (in 2010) of the seven study sites in the different successional stages: (1) 0 years, (2) 10 years, (3) 25 years, (4) 35 years, (5) 45 years, (6) 55 years, (7) 100 years in 2010. Different isoclines represent the different stages of vegetation colonization (until 1997). The chronosequence map was constructed using topographical maps and aerial pictures (methods described in Olff and others 1997; De Jager 2006).

## Estimations of Marine Input

Both bird guano and macro-algae that drift ashore can be an important input of nutrients (Polis and others 1997; Maron and others 2006) and estimations were made of the input of both sources of nutrients. Macro-algal dry weight was estimated in March and April 2010 by collecting all macro-algae from each 5 m × 5 m plot, which were subsequently rinsed, and dried at 70°C for 48 h. Afterwards, samples were weighed to determine dry weight per square meter. Bird densities were estimated by weekly counts of the numbers of resting birds at all sampling sites during the months March and April. Every location was visited four times. At every site, we used one 1-ha plot in which the total number of resting birds per species was estimated between 1 h before and 3 h after high tide. Per species, the total number of bird minutes was calculated by multiplying the number of resting birds at every location times the number of minutes spend in each of the hectare plots.

## Sampling for Isotope Analysis

### *Terrestrial Plants*

At each plot location, we collected ten leaves from ten different individual plants for each of the dominant plant species. The dominant plant species comprised at least 90% of the locally produced biomass. To standardize samples, only fresh plant leaves were collected. Stable isotope analysis of plant leaves were done on samples from succession stages 0, 10, 45, and 100 years, because we expected no strong differences in isotopic signals between sites. At each plot, a sample of local litter material was collected by taking five random samples of 10 g of dead local vegetation, which were not overgrown with algae or covered in clay. These samples were well-mixed in a bowl from which a subsample of about 5 g was taken for analysis.

### *Marine POM*

Because no flooding events occurred during the sampling period, we sampled marine particulate organic matter (POM) at high tide at a distance of 150 m south of succession stages 10, 45, and 100 years. At five points per site, spaced 25 m apart, we used a plastic hand-held net (30 cm × 30 cm) with fine mesh (500 µm) which was manually moved five times over a distance of a meter through the upper 50 cm of the water column to catch drifting material. The content of the net was deposited on a clean plastic sheet and funnelled into a 2-ml plastic tube with demi water and stored at −20°C. Because

we expected no large differences for marine POM stable isotope signals for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  between sampling locations, no additional sampling was done in other succession stages.

### *Diatoms*

As marine diatoms represent a significant fraction of the organic matter that is deposited during floodings (Boschker and others 1999), we made collections of diatoms at locations close to the plots. Diatoms were collected from the marine sediment at low tide at a distance of 150 m south of succession stages 0, 10, 45, and 100 years. The five sampling plots per site were spaced 25 m apart. From each of the five samples per site, we took one sediment sample of 10 cm × 10 cm × 1 cm deep (100 cm<sup>3</sup>) from which diatoms were extracted in the laboratory. To separate the diatoms from the sediment, we applied the 'lens-tissue method' (Eaton and Moss 1966). After the diatoms migrated onto a GF/F Whatman filter (average pore size = 0.7 µm), the content was deposited on a plastic sheet and funnelled into a 2-ml plastic tube using demineralized water and stored at −20°C. As we expected no strong differences between sites for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotope signals, we did not sample any of the other sites.

### *Marine Macro-algae*

We collected the macro-algae *Fucus* spp. because this species comprises the majority of the deposited marine macro-algae (>90%, see Table 1). Samples were collected in all 5 m × 5 m plots at succession stages 0, 10, 45, and 100 years. Every sample contained five random leaves of individual *Fucus* spp. deposits, which were at least 2 m apart. After collection, all samples were brought to the laboratory where they were carefully rinsed with demineralized water to remove other organic material and mineral sediments and stored in 2 ml tubes at −20°C.

### *Invertebrates*

Dominant invertebrate species (identified as dominants in Schrama and others (2012)) were collected in all subplots, using a modified leaf blower (Echo Shred 'N' Vac, net build inside with a mesh size of 1 mm). After applying the leaf blower for 2 min in each 5 m × 5 m plot, the net was emptied in a white plastic container and living specimens were collected by hand and put into 2 ml plastic tubes. In addition to sampling with the leaf blower, we used hand collections to sample the amphipod

**Table 1.** Resting Marine Birds (in bird min h<sup>-1</sup> ± SD) and Amount of Marine Macro-algal Input (mg m<sup>-2</sup> month<sup>-1</sup>) in Each of the Stages of Succession

Bird name	Number of bird minutes per hour per hectare succession stage (years)													
	0	SD	10	SD	25	SD	35	SD	45	SD	55	SD	100	SD
Oystercatcher ( <i>Haematopus ostralegus</i> )	32,572	30,840	40	44	0	0	0	0	0	0	0	0	0	0
Dunlin ( <i>Calidris alpina</i> )	1,800	2,080	692	760	0	0	0	0	0	0	0	0	0	0
Brent goose ( <i>Branta bernicla</i> )	452	520	420	460	0	0	0	0	0	0	0	0	0	0
Herring gull ( <i>Larus argentatus</i> )	152	172	20	20	0	0	0	0	0	0	16	16	0	0
Black-headed gull ( <i>Chroicocephalus ridibundus</i> )	60	68	0	0	0	0	0	0	0	0	0	0	0	0
Eider duck ( <i>Somateria mollissima</i> )	0	0	360	396	0	0	0	0	0	0	0	0	0	0
Grey plover ( <i>Pluvialis squatarola</i> )	0	0	20	20	0	0	0	0	0	0	220	200	0	0
Lesser black-backed gull ( <i>Larus fuscus</i> )	0	0	40	44	0	0	0	0	0	0	0	0	0	0
Redshank ( <i>Tringa totanus</i> )	0	0	32	36	0	0	0	0	0	0	0	0	0	0
Total marine birds	32,572	30,840	1,624	1,304	0	0	0	0	0	0	236	216	0	0
Marine macro-algae (mg m <sup>-2</sup> month <sup>-1</sup> )	594	223	797	196	5	3	2	1	2	0	3	0	6	1

Bird minutes were calculated by multiplying the number of birds times the number of minutes birds spend in each hourly observation in each hectare plot.

*Orchestia gammarellus*, Enchytraeds and the snail *Ovatella myositis*. Each sampling tube contained at least four individuals of each species. After collection, all tubes were stored at -20°C. Literature and personal observations on feeding preference were used to assign species to different trophic groups: herbivores, detritivores, herbivore-feeding carnivores, and carnivores feeding on both herbivores and detritivores (hereafter called omnivorous carnivores). In total, 10 species of invertebrate herbivores were collected, but not the same species from all succession stages. In total 14 species of carnivores were collected. The group of carnivores feeding on herbivores and detritivores contained mostly spiders (Erigonidae and Lycosidae), beetles (Carabidae) and ants (Formicidae). Carnivores that fed on herbivores were different species of ladybugs (Coccinellidae) and parasitoid wasps (Ichneumonidae). In total 10 species of detritivores were collected, mainly Amphipoda, Isopoda, and beetles. Because many detritivore species cover the whole spectrum of food sources and because little is known about their exact feeding preference, no a priori subdivision of this group was made.

#### Marine Birds

Because oystercatchers (*Haematopus ostralegus*) and lesser black-backed gulls (*Larus fuscus*) were found to be dominant roosting birds at the first successional stage, five samples of fresh feces of both bird species were collected in five 25-m spaced 3 m × 3 m plots. These plots were located 50 m south of the plots at which the other collections

were done, but only at the first successional stage. Per species per sample we took one individual pallet of guano from 5 individual birds, directly after defecation, using a pair of tweezers.

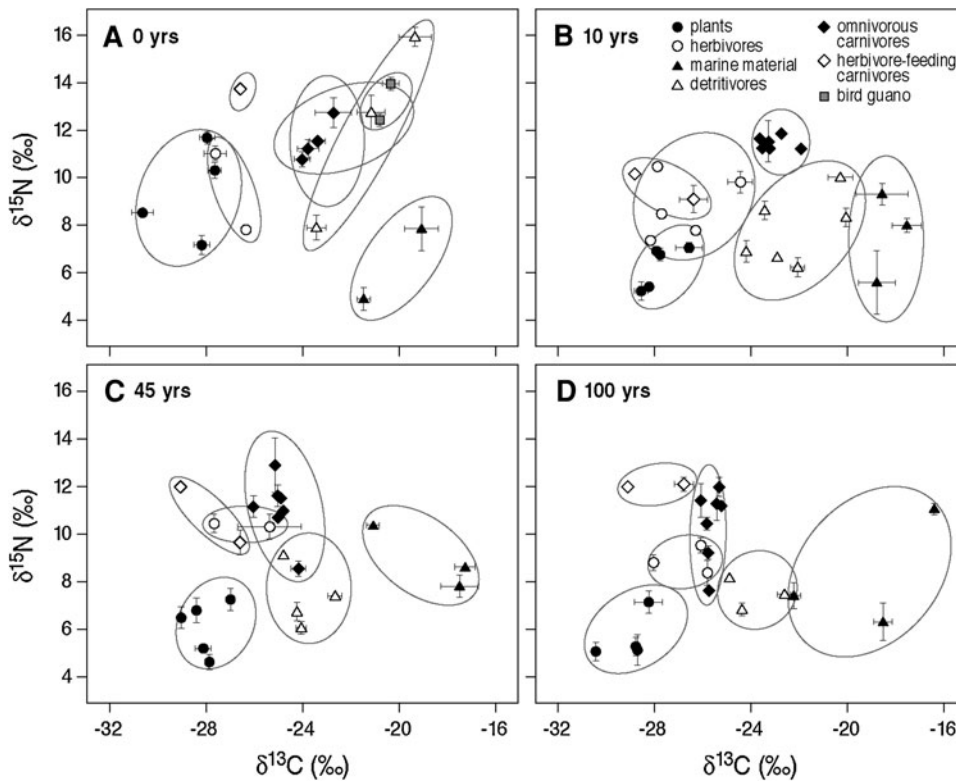
#### Stable Isotope Analysis

All invertebrate, plants, and marine macro-algae samples were stored frozen at -20°C and processed by freeze-drying and grinding with a pebble mill (1000 rotations per minute for 2 min using a Retsch MM2). The δ<sup>13</sup>C and δ<sup>15</sup>N isotopes values were determined by using a Thermo Flash 2000 elemental analyzer coupled to a Thermo Delta V isotope ratio mass spectrometer. Isotope values were calibrated to a laboratory acetanilide standard (δ<sup>13</sup>C -26.1‰ and δ<sup>15</sup>N 1.3‰ calibrated on NBS-22 and IAEA-N1, respectively) and corrected for blank contribution. The samples were mostly analyzed in duplicate and the reported data represent the mean of these analyses. The results are reported on the per mille scale with respect to Vienna Pee Dee Belemnite (VPDB) and graphically presented in δ<sup>13</sup>C and δ<sup>15</sup>N isotopes diagrams, to visualize changes in carbon and nutrient sources and trophic structure during succession. The results were graphically presented in isotope ratio diagrams to analyze changes in carbon and nutrient sources and trophic structure during succession.

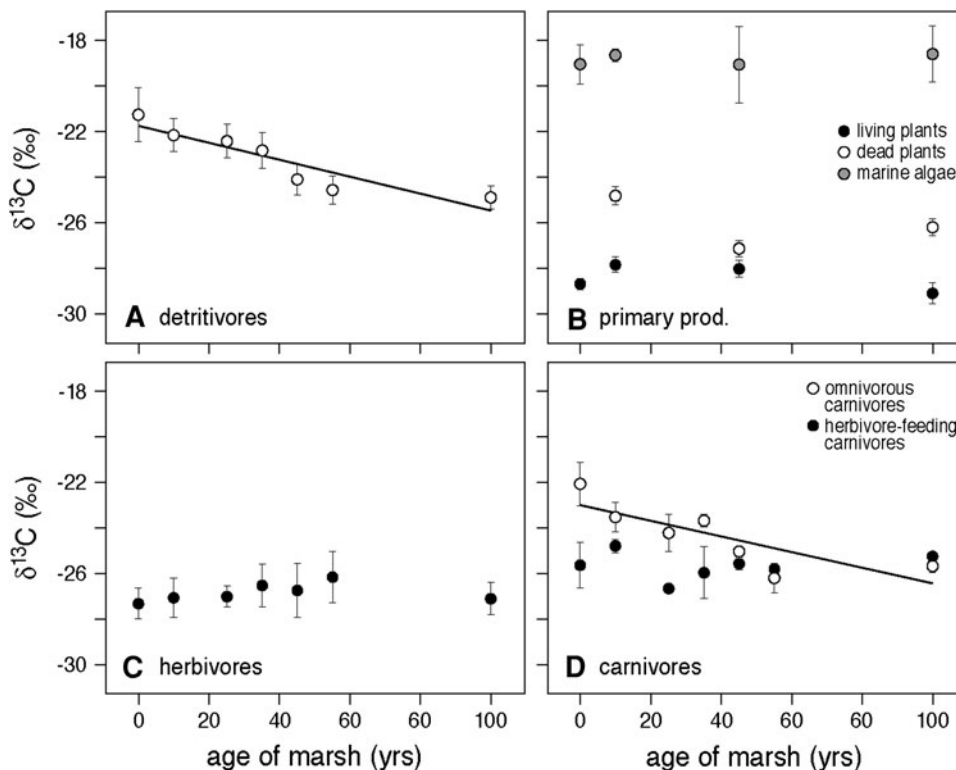
#### Statistics

For the analysis of changes in food web stable isotopes composition estimations for species were averaged within trophic groups. To test for differences





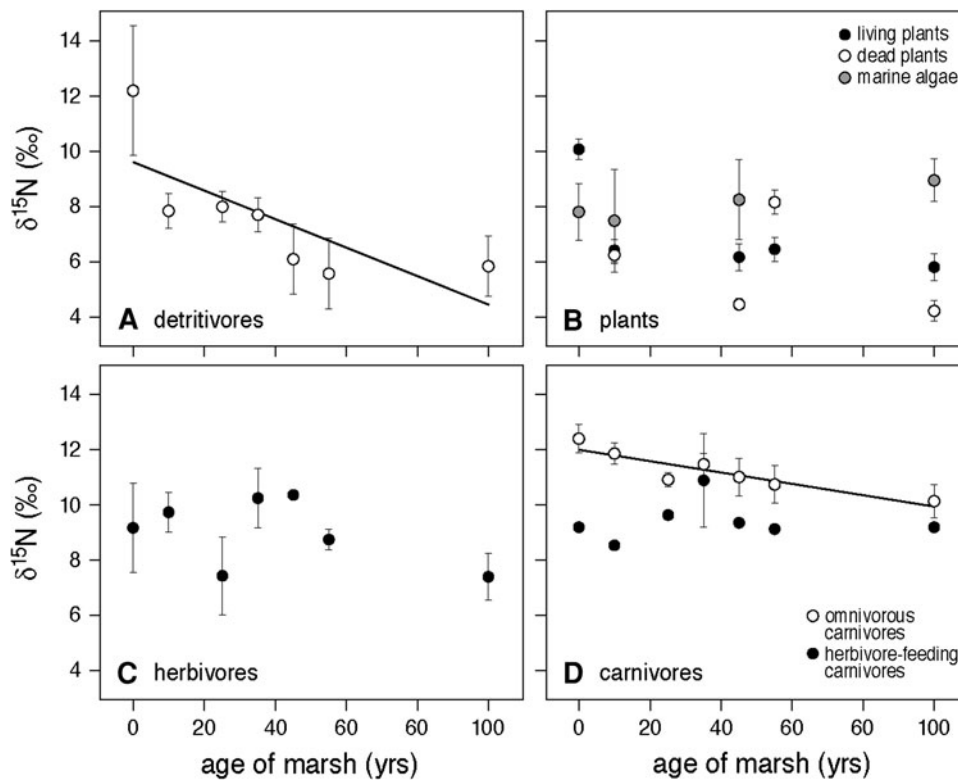
**Figure 2.** All species in a C–N plane depicted in four succession stages: **A** 0 years, **B** 10 years, **C** 45 years, and **D** 100 years. Circles with different shadings represent different trophic groups, indicated in the legend. Note that primary producers show only very small shifts on the carbon-axis over time. Mark the widening gap between marine primary producers and other trophic groups (particularly carnivores and detritivores) over successional time.



**Figure 3.**  $\delta^{13}\text{C}/\delta^{12}\text{C}$  values for all trophic groups. Symbols show averages  $\pm$  SE. Groups belonging to the brown web are depicted with open circles; groups belonging to the green web are depicted with black circles. **A** Detritivores (linear regression:  $R^2 = 0.41$ ;  $P < 0.001$ ), **B** marine and terrestrial primary producers, **C** herbivorous invertebrates, **D** herbivore-feeding carnivores and carnivores feeding both on herbivores and detritivores (linear regression:  $R^2 = 0.66$ ;  $P = 0.02$ ).

between trophic groups or within trophic groups between succession stages, general linear models with post hoc Tukey HSD tests were done as

assumptions needed for doing parametric analysis were met. Linear least square regressions were done within each trophic group to find changes in



**Figure 4.**  $\delta^{15}\text{N}/\delta^{14}\text{N}$  values for all four trophic groups. Symbols show averages  $\pm$  SEM.

**A** Detritivores (linear regression:  $R^2 = 0.48$ ;  $P < 0.05$ ), **B** terrestrial and marine primary producers, **C** herbivores, **D** carnivores feeding on herbivores and carnivores feeding both on herbivores and detritivores (linear regression:  $R^2 = 0.81$ ;  $P < 0.005$ ). Note the high value for  $\delta^{15}\text{N}$  in the first succession stage for both **A** and **B**.

stable isotope ratios for both nitrogen and carbon over succession, using Statistica 9.0.

## RESULTS

### Food Web Composition

Plotting all species in the various plant successional stages in a  $\delta^{15}\text{N}$ – $\delta^{13}\text{C}$  plane reveals major changes in food web structure (Figure 2A–D). A strong difference between  $\delta^{13}\text{C}$  values of salt marsh plants and marine diatoms was observed, of about 10‰ of  $\delta^{13}\text{C}$  on average.

On average herbivores had 2–3‰ higher  $\delta^{15}\text{N}$  values than primary producers (Figure 2A, C). Also, the  $\delta^{15}\text{N}$  values for carnivores were 2–3‰ above values for herbivores and 3–4‰ above detritivores, except in the first stage of succession.

### From Early to Intermediate Succession

To obtain insight on how various trophic groups that either belong to the green web (terrestrial plants and herbivores) or the brown web (detritus and detritivores) are fuelled during early succession and how this changes towards intermediate succession stages, we graphed trends of average  $\delta^{13}\text{C}$  signatures (Figure 3, mostly reflecting changes in base levels) and  $\delta^{15}\text{N}$  (Figure 4, mostly reflecting trophic changes) over time. Differences between

$\delta^{13}\text{C}$  levels of the green web and the brown web were greatest during early succession (Figure 3A–D). The low carbon isotopic value for detritivores ( $-21.2 \pm 0.42$ ) at the start of succession is much lower than for herbivores ( $-27.4 \pm 0.21$ ; Tukey HSD;  $N = 5$ ;  $P = 0.002$ ) and herbivorous carnivores ( $-26.0 \pm 0.9$ ; Tukey HSD;  $N = 5$ ;  $P < 0.05$ ). This suggests that detritivores derived their energy initially mostly from marine sources. Marine sources such as diatoms, bird guano, and marine macroalgae had average  $\delta^{13}\text{C}$  levels between  $-21$  and  $-18$ ‰ and were most abundant in early succession. Numbers of resting marine birds and input of marine macroalgae (isotope value) were highest in the first two stages of succession (Table 1), with oystercatchers (*Haematopus ostralegus*) making up the majority of resting birds. Interestingly, omnivorous carnivores (that is, feeding on herbivores, detritivores, and other carnivores, such as spiders) had similar elevated levels of  $\delta^{13}\text{C}$  to those of detritivores ( $21.2 \pm 0.42$  vs  $-23.0 \pm 0.52$ , Tukey HSD;  $N = 5$ ;  $P > 0.5$ ), whereas herbivore-feeding carnivores, such as ladybugs resembled herbivore  $\delta^{13}\text{C}$ -values in these early stages of succession ( $-27.4 \pm 0.21$  vs  $-26.0 \pm 0.9$ ; Tukey HSD;  $N = 5$ ;  $P > 0.5$ ). This suggests that the omnivorous carnivores were mostly feeding on the marine-subsidized detritivores during this stage of succession.



We found very high  $\delta^{15}\text{N}$  values for detritivores ( $\delta^{15}\text{N}$   $12.5 \pm 0.2$ ) during the first stage of succession (Figure 4A–D), which were especially high for Enchytraeids ( $\delta^{15}\text{N}$  levels between 15.2 and 16.5, Figure 2A). This again suggests that at least some dominant species in this trophic group feed on high trophic marine sources ( $\delta^{15}\text{N}$  values  $5.6 \pm 1.1$  for macro-algae and  $8.6 \pm 0.2$  for diatoms vs  $13.0 \pm 0.3$  for bird guano). Omnivorous carnivores ( $\delta^{15}\text{N}$   $11.5 \pm 0.2$ ) also yielded high levels of  $\delta^{15}\text{N}$  during early succession, indicating again a trophic link between omnivorous carnivores and the detritivore part of the web. Interestingly, the few terrestrial plant species that we sampled in the earliest successional stage also had slightly elevated levels of  $\delta^{15}\text{N}$  compared to the same plant species in the next stage of succession (Tukey HSD;  $N = 5$ ;  $P < 0.001$ ; Figure 4B), which indicates that their nitrogen may have come from marine sources.

### From Intermediate to Late Succession

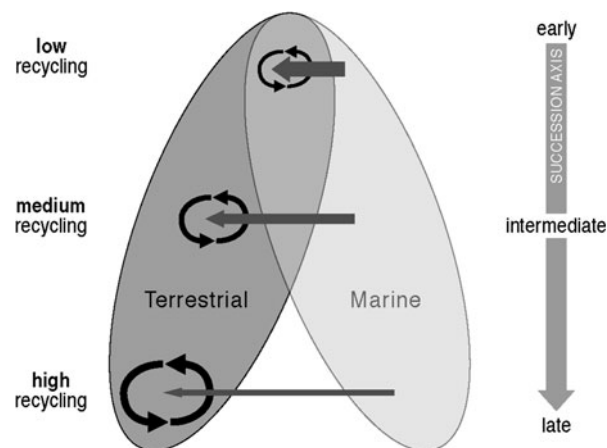
For herbivores, we observed no change in  $\delta^{13}\text{C}$  between early and late successional stages, whereas detritivores and omnivorous carnivores showed a strong decrease in  $\delta^{13}\text{C}$  isotopic signal (Detritivores:  $R^2 = 0.41$ ,  $P < 0.001$ , Figure 3A; Omnivorous carnivores:  $R^2 = 0.66$ ,  $P = 0.02$ , Figure 3D). Patterns for  $\delta^{15}\text{N}$  were similar, but somewhat less pronounced. Both detritivores and omnivorous carnivores showed a gradual and significant decrease in  $\delta^{15}\text{N}$  levels over succession (Detritivores:  $R^2 = 0.48$ ,  $P < 0.05$ , Figure 4A; Omnivorous carnivores:  $R^2 = 0.81$ ,  $P < 0.005$ , Figure 4D), whereas herbivores and herbivore-feeding carnivores remained stable over succession (Figure 4C, D). Both marine and terrestrial primary producers had the same  $\delta^{15}\text{N}$  signal in the later stages of succession. The clear observed differences in  $\delta^{13}\text{C}$  signal between herbivores and detritivores during early succession disappeared towards later successional stages. This was accompanied by a gradual replacement in detritivore species. Although in early succession *Fucellia maritima* and Enchytraeid worms are dominant, beach hoppers (*Orchestia gammarellus*) comprise most of the detritivore biomass in later stages of succession.

### DISCUSSION

Our results strongly suggest that during this primary succession over a period of more than 100 years, the energy and nutrient sources fuelling this terrestrial food web changed from mostly driven by external, marine inputs towards a dependence on internal nutrient cycling (Figure 5). Both macro-detritivores

and omnivorous carnivorous invertebrates (that is, the brown food web component) showed a remarkable shift in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotope values with succession, whereas other components of the food web, such as plants and their herbivores (that is, the green web food component) remained more similar in this respect. This pattern is likely caused by both a decline in marine inputs during succession and an increase in nutrient pools and nitrogen mineralization due to the development of a litter layer as observed in earlier work (Van Wijnen and Bakker 1999). So, the role of the brown web along this successional chronosequence changes from being vectors of external nutrients in early succession towards agents of internal cycling in late succession, whereas the green part of the food web remains dependent on local production all along the successional gradient.

Several other studies have shown that the successional dynamics on Schiermonnikoog are highly comparable to those on other natural salt marshes in North Western Europe (Bakker and others 1993;



**Figure 5.** Conceptual overview of changes in nutrient supply to the food web over a gradient of successional stages. The two circles depict two ecosystems: the intertidal marine and the terrestrial salt marsh ecosystem. Dark grey arrows indicate the magnitude of nutrient flow from the marine towards the terrestrial ecosystem and circular black arrows indicate the magnitude of local nutrient cycling. We distinguish three distinct phases during succession: early, intermediate, and late succession. In early succession, external (marine) input of nutrients supports a brown web (detritus-detritivore dominated, indicated by a thick grey straight arrow from marine to terrestrial) with low internal production. Intermediate succession is characterized by lower external input of nutrients and a fairly high local production, which supports both a green (plant-herbivore dominated) and a brown web. Late succession is characterized by low external input of nutrients, high internal cycling and low quality plant material, which results in a brown-web dominated state.

De Leeuw and others 1993; Olff and others 1997; Kuijper and others 2003). This suggests that our results on the food web dynamics in the different stages of succession are likely to represent a general pattern and that similar patterns in food web assembly can be found on other natural marshes. Recently, it was shown that the contribution of marine carbon in the diets of invertebrates declines across the sea-land axis in dune ecosystems (Colombini and others 2011), which is another indication that this may be a general pattern for many more ecosystems.

Moreover, we expect that our sampling along the chronosequence accurately captured the temporal patterns of the food webs dynamics. We expect that measuring in a different season would not change the qualitative nature of the results because (a) the signal for the stable isotopes in the tissues of invertebrates yields an integrated account of the feeding patterns, which gives a much better temporal integration than for instance gut content analysis (Post 2002); (b) most species only have one generation time per year with a pronounced peak in July, owing to a relatively short growing season (Irmeler and Heydemann 1986). It is nevertheless conceivable that the marine signal for species in the brown web would be stronger if samples were collected shortly after a flooding event (or in winter). However, this would only change the results in a quantitative way because we would still expect the highest relative marine input in the earliest successional stages. We therefore expect that our main conclusions will hold upon more detailed analysis at other locations, in other ecosystems and in different seasons.

### What Causes Succession to Start?

The main sources of energy and nutrients for the food web assembly in the first stage of succession shows many similarities to the Baja California islands that were described by Polis and Hurd (1995). Both have a high marine input, a low local primary production and a high abundance of invertebrates that do not rely on locally produced organic material, but rather on detritus. Peak standing biomass of living vegetation in our earliest stage varies between 5 and 50 g m<sup>-2</sup> and covers only up to 2% of the soil surface, whereas in later stages of succession peak standing biomass is between 830 and 1050 g m<sup>-2</sup> (Schrama and others 2012). A difference, however, between our first succession stage and the desert islands described by Polis and Hurd (1995), is the much higher rainfall and nutrient inputs by inundations and guano from waders at our study site,

which allows the onset of long-term succession towards dense vegetation.

Therefore, we hypothesize that nutrients that enter in the first stage of succession operate as a 'kick-start' to long-term food web assembly. Which of the different components of marine subsidy, that is, guano, macro-algae, diatoms, or carrion is the most important source for fuelling the food web at this stage of succession was not the focus of our study. However, our bird count data suggest that nutrients from bird guano may play a very important role in starting vegetation succession and associated food web assembly. Using data from Zwarts and Blomert (1996) on fecal nutrient concentrations of marine birds, combined with our observed densities, we can roughly estimate that the first stages of succession may receive up to 30 kg of N per ha per year, whereas macro-algae yield only 8–10 kg N ha<sup>-1</sup> y<sup>-2</sup>. As the main bird species responsible for this are non-migratory oystercatchers, this input is relatively constant throughout the year, not limited to specific seasons. Furthermore, this nitrogen input by high trophic level marine-feeding predators provides a good explanation for the relatively heavy isotope signals that we found for early successional plants and detritivores. So, we conclude that our earliest successional stages can be seen as marine-subsidized food webs, both at the trophic level of the primary producers as well as on higher trophic levels.

### Causes of Declining Marine Inputs

The suggested reduction of marine inputs towards later successional stages is likely caused by a combination of factors. Firstly, waders and gulls have high tide roosts in the sparsely vegetated zones that surround the mudflats, where the primary successional stages are also located (pers obs. MJJS, MPB; Rogers 2003). Secondly, a 'sieving effect' of taller vegetation in older stages at the direct edge of the salt marsh prevents the sedimentation of marine material onto the marsh at high tides, where larger organic matter such as macro-algae are sieved out first (Temmerman and others 2005). However, this may imply that our estimation of the deposition of marine subsidy provides an underestimation of the total amount of marine subsidy, especially of the small organic matter fraction. This unknown POM fraction may be more important for explaining successional food web dynamics than we originally anticipated, especially in the intermediate successional stages (up to 35 years). Two lines of argument support this hypothesis. First, the thickness of the sediment layer (which is a mix of clay particles and small POM (Olff and others 1997)) increases

steeply towards the 35- to 45-year-old stage (Electronic appendix, Table 1), after which it levels off. Secondly, we find that the  $\delta^{13}\text{C}$ -signature for both detritivores and carnivores shows a less strong marine signal after 35–45 years of succession. To what extent small organic matter is indeed more important in sustaining the (brown) web than the larger fractions of organic material deserves further attention.

The consequences of the observed decrease in external nutrient inputs of marine origin are most clearly observed in the isotopic signal for detritivores and omnivorous carnivores. Although in early succession these groups exhibit a strong external (marine) signal, the diminishing external marine input—not only in relative but also in absolute terms—causes the brown part of the food web to rely more on the local production of plant litter as succession proceeds. Local plant biomass production is high in late successional stages and so is local litter input (Schrama and others 2012; Olff and others 1997). Detritivore species now have isotopic signals similar to plants, carnivores, and herbivore species, which indicates that both the green and the brown part of the food web mainly use terrestrially produced organic matter as a source of nutrients. The increase in N mineralization towards later successional stages is driven by the enhanced total pool of soil nutrients which accumulates during succession due to vegetation development, a cumulative effect of more nutrient inputs than losses during all stages (Olff and others 1997; Van Wijnen and Bakker 1999). Therefore, we conclude that the combined decrease in the input of marine, external organic material and an increase of the primary production causes both the green and brown parts of the food web to become increasingly ‘fuelled’ by local primary production towards late successional stages.

### The Role of Carnivorous and Detritivorous Invertebrates in Early Succession

From other studies on primary succession it is known that spiders and ground beetles are amongst the first carnivorous species to invade an area (Hodkinson and others 2001; Kaufmann 2001; Coulson and others 2003). We also observe this in our salt marsh food web assembly, where omnivorous carnivores and detritivores thrive on external inputs in the earliest successional stages. On early successions at glacier forelands and volcanic sediments, external inputs of windblown insects are also an important organic source for early food web assembly (Edwards

and Sugg 1993; Coulson and others 2003). This will also happen in our system, but we suggest that on salt marshes the importance of this input is minor compared to marine nutrient inputs. Although the relative importance of local primary production versus the external input of energy and nutrients as a food source for these early soil dwelling carnivores and detritivores has seldomly been addressed, our findings emphasize the importance of external marine inputs of nutrients and energy to the first stage of succession. This upsets the idea further that colonizing plants are required to ‘get nutrient cycling and vegetation succession going’.

### Consequences of Declining Detritus Quality

The quality of the coarse detritus that is decomposed by the brown part of the food web in early succession is likely much higher than the later stages (Olff and others 1997), even though both stages of succession are dominated by members of the brown web (Schrama and others 2012). Enchytraeid worms and *Fucellia maritima* are dominant species in the earliest succession stage, where the high deposition of marine derived organic material is likely to be of higher quality than the terrestrial detritus in the later successional stages (Shurin and others 2006). The intermediate to later stages of succession have increasingly larger stocks of decreasing quality of organic matter (Van der Wal and others 2000; Kuiper and others 2004). This decline in litter quality is most likely driven by increasing importance of light competition among plants, which results in dominance of grass species with high investments in structural tissues of the dominant plant species (Huisman and Olff 1998) and resulting low litter quality (Olff and others 1997). The macro-detritivores in this part of the salt marsh consist almost entirely of the species *Orchestia gammarellus*, a semi-terrestrial amphipod. Its litter processing as well as its digging behavior make litter more easily accessible and decomposable for bacteria and fungi (Moore and Francis 1986). We suggest that because of the lower quality of litter in late succession, litter preprocessing by these macro-detritivores may be essential for nutrient mineralization. So, where the first stage of succession resembles a marine food web, with high quality organic material, high turnover of organic matter and low standing plant biomass (Cebrian and Lartigue 2004; Shurin and others 2006), it gradually changes into a typical terrestrial food web with lower organic matter quality, slower cycling of nutrients and higher standing plant biomass.

## CONCLUSIONS

We suggest that our findings represent a general pattern of ecosystem assembly, which is schematically represented in Figure 5. The earliest successional stages are subsidized with external, high quality organic material which 'kick starts' early successional vegetation development. After the initial kick-start, internal cycling of nutrients becomes progressively more important, where the brown part of the food web increasingly depends on this internal cycling of nutrients. The amount of subsidy of organic matter will determine the subsequent rate of food web assembly. From several other ecosystems it is known that primary succession is happening much slower, which is likely caused by lower initial inputs. However, several recent studies on primary succession (Sugg and Edwards 1998; Kaufmann 2001; Hodkinson and others 2004), now seem to agree on the importance of some form of external input of nutrients to the system to initiate long-term developments. For our ecosystem we conclude that 100 years of food web assembly proceeds by gradual decoupling of terrestrial nutrient cycling from the marine environment, by associated rearrangements in energy channels between the brown and green part of food webs, and increasing importance of macro-detritivores for nutrient cycling during later successional stages. Food web assembly thus interacts with nutrient and energy flows across ecosystem boundaries.

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